PATENT
Attorney Docket No. 275.00100101

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	David MUNN *et al.*	Group Art Unit:	1614
Application No.:	10/780,797	Confirmation No.:	1508
Filed:	February 17, 2004	Examiner:	James D. Anderson

FOR: REGULATION OF T CELL-MEDIATED IMMUNITY BY D ISOMERS OF INHIBITORS OF INDOLEAMINE-2,3-DIOXYGENASE

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DECLARATION OF DR. GEORGE C. PRENDERGAST

I, George C. Prendergast, hereby declare that:

1. I am a President and CEO of the Lankenau Institute for Medical Research, located west of the city of Philadelphia, Pennsylvania. I hold a Ph.D. in Molecular Biology, received from Princeton University in 1989. My career as a cancer researcher includes training as an American Cancer Society Postdoctoral Fellow at the Howard Hughes Medical Institute at NYU Medical Center, working at the Department of Cancer Research at Merck Research Laboratories, appointment as Assistant Professor and later Associate Professor at the Wistar Institute, and experience as a Senior Director of the Cancer Research Group at the DuPont Pharmaceutical Company. I have published over 100 peer-reviewed research reports, and I am an inventor or co-inventor on 23 patents or patent applications. I am familiar with scientific progress and various developments in the field of cancer research. Therefore, I can be considered an expert in the field of cancer research.

2. The purpose of this Declaration is to provide factual evidence that 1-D-methyl-tryptophan (D-1MT) can be effective in inhibiting cancer growth in multiple cancer types.

3. Attached as Exhibits A and B are graphs presenting data, which demonstrate the anti-cancer effects of the administration of D-1MT in conjunction with a chemotherapeutic agent in a treatment regimen against lung cancer and colon cancer, respectively.

4. The data presented in Exhibit A was obtained as follows. C57/Bl6 mice were injected with $1x10^6$ LLC1 lung tumor cells subcutaneously (SC) on day 1. On day 7, 400 mg/kg of D-1MT was administered perorally (PO) twice daily (BID) Monday through Friday throughout the length of the experiment. On days 9, 11, and 14, 125 mg/kg of cyclophosphamide (CTX) was injected intraperitoneally (IP). Tumor volume was measured on days 15, 18, and 22. As shown by the data in Exhibit A, the administration of D-1MT with CTX effectively inhibited lung cancer growth, and the effect was greater than the effect of either compound administered individually or the combined effect of the compounds administered individually.

5. The data presented in Exhibit B was obtained as follows. Balb/C mice were injected with $1x10^6$ CT26 colon tumor cells SC on day 0. On day 7, 400 mg/kg of D-1MT was administered PO twice daily (BID) Monday through Friday throughout the length of the experiment. On days 9 and 11, 125 mg/kg of CTX was injected IP. Tumor volume was measured on days 15, 18, and 22. As shown in the data presented in Exhibit B, the administration of D-1MT with CTX effectively inhibited colon cancer growth, and the effect was greater than the effect of either compound administered individually or the combined effect of the compounds administered individually.

I hereby declare that all statements made herein of my own knowledge are true, and that all statements made based on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful statements may jeopardize the validity of the above-referenced application or any patent issued therefrom.

9/23/08
Date

George C. Prendergast, Ph.D.

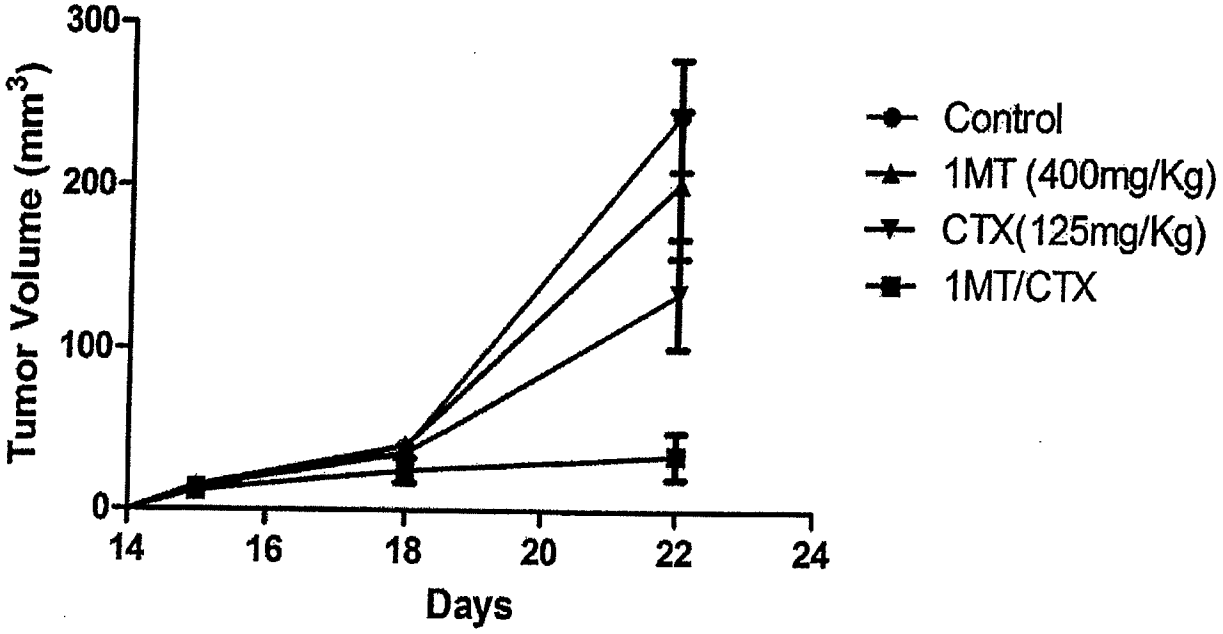
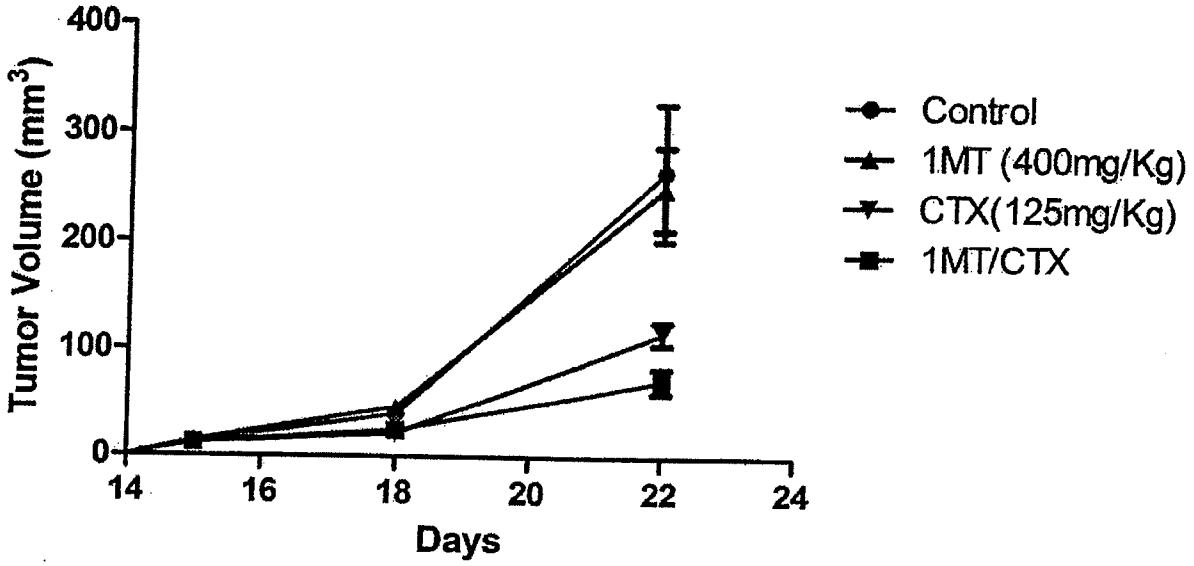

300
200
100
0
Tumor Volume (mm3)
14
16
18
20
22
24
Days
Control
1MT (400mg/Kg)
CTX(125mg/Kg)
1MT/CTX

Exhibit A


400
300
200
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Tumor Volume (mm3)
14
16
18
20
22
24
Days
Control
1MT (400mg/Kg)
CTX(125mg/Kg)
1MT/CTX

Exhibit B

EXHIBIT C

Med Chem Res (1994) 3:531–544

MEDICINAL CHEMISTRY RESEARCH

EVALUATION OF FUNCTIONALIZED TRYPTOPHAN DERIVATIVES AND RELATED COMPOUNDS AS COMPETITIVE INHIBITORS OF INDOLEAMINE 2,3-DIOXYGENASE1

Andrew C. Peterson*, Michael T. Migawa, Michael J. Martin, Linda K. Hamaker, Kevin M. Czerwinski, Weijiang Zhang, Richard A. Arend[a], Philip L. Fisette[a], Yoshisuke Ozaki[a], James A. Will[b], Raymond R. Brown[a] and James M. Cook*

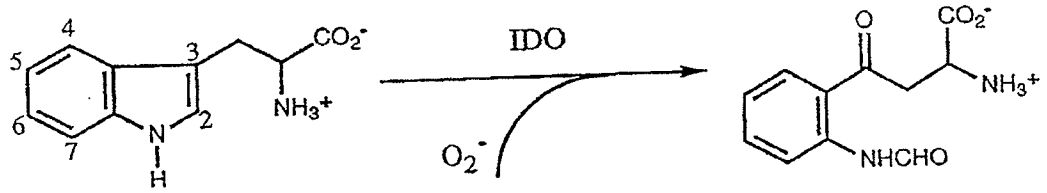
Department of Chemistry, University of Wisconsin-Milwaukee, Milwaukee, Wisconsin 53201 and [a]*Medical Science Center, University of Wisconsin Medical School, Madison, Wisconsin 53706 and* [b]*Department of Veterinary Science, University of Wisconsin-Madison, Madison, Wisconsin 53706.*

Abstract. The stereochemical requirements of the active site of human monocyte/macrophage IDO were probed by assays of both antipodes of 1-methyltryptophan **3a/3b**. The L-isomer of 1-methyltryptophan **3a** (62.9% inhibition, $K_i = 34\ \mu M$) was found to be significantly more active than its antipode **3b** (11.6% inhibition). These results indicate stereoselectivity of binding at the tryptophan binding site of IDO. The 1-ethyltryptophan antipodes **4a/4b**, however, were not potent inhibitors of this enzyme. In addition, both D- and L-dihydrotryptophan diastereomers **7** and **8** were found to be inactive. In contrast, both DL-oxindolylalanine **9** and 5-methoxytryptamine **26** were relatively potent effectors, activating IDO at 43.3% and 43.9% activation, respectively. Quinolinic acid **31**, kynurenic acid **34**, L-kynurenine **32**, 3-hydroxykynurenine **33**, xanthurenic acid **35** and picolinic acid **30** were all inactive. However, L-5-hydroxytryptophan **36** showed modest inhibition (14% inhibition). The D- and L-isomers of quinoline-3-alanine **16** and **17**, respectively, were inactive as were tryptazan and DL-7-azatryptophan **10** and **11**. These results suggest that the tryptophan binding site of IDO is composed of a largely lipophilic cleft, except in the region where the amino acid function binds. The nonindolic compound, 3-amino-2-naphthoic acid **19**, was found to be the most potent inhibitor in this investigation (75.2% inhibition) and demonstrates that the indole nitrogen moiety is not necessary for activity at this site.

Introduction.

In mammals L-tryptophan and other indoleamines are oxidized to formylkynurenines *via* the kynurenine pathway by indoleamine 2,3-dioxygenase (IDO) or tryptophan 2,3-dioxygenase (TDO).[2] Although both enzymes catalyze the same transformation, TDO is found only in the liver[3] while IDO is present in a wide variety of tissues, such as the brain, lung and small intestine.[4] Furthermore, IDO is a 41 kD, monomeric heme containing enzyme[5] that utilizes superoxide to cleave the 2,3-double bond of indoleamines.[6] The conversion of tryptophan **1** into kynurenine **2** by IDO is depicted in Scheme 1.

Scheme 1



1 **2**

A mechanism for this transformation has been proposed and is analogous to that proposed for the reaction of singlet oxygen with tryptophan.[7]

Many inflammatory diseases and neurodegenerative diseases have been hypothetically linked to aberrant L-tryptophan metabolism caused by activation of IDO.[8] However, Heyes and coworkers have recently reported evidence which implicates the activation of IDO only in inflammatory diseases, such as acquired immune deficiency syndrome (AIDS), meningitis and sepcis.[9] Interferon-γ has been shown to induce the production of IDO.[10] A protein which is induced by interferon-γ may induce the transcription of IDO.[11] One of the ways in which the body responds to infection by foreign organisms and head injuries is to produce large amounts of interferon-γ and other immune system activators. High levels of interferon-γ eventually induce the production of high levels of IDO which result in the catabolism of large amounts of L-tryptophan and the production of high levels of metabolites of the kynurenine pathway.

A variety of bioactive metabolites are produced through the kynurenine pathway; the most significant of these are kynurenic acid, quinolinic acid and nicotinamide adenine dinucleotide (NAD). Quinolinic acid has been shown to be an agonist for the excitatory amino acid receptors of the N-methyl-D-aspartate (NMDA) receptor-ion channel complex.[12] When present in high levels quinolinic acid causes many neuropathological symptoms including nerve cell death and dementia.[13] High levels of quinolinic acid are marked by the formation of brain lesions.[14] Recently, only macrophage-

derived cells and certain liver cells have been shown capable of the synthesis of quinolinic acid from labelled L-tryptophan following immune stimulation.[15] Large amounts of quinolinic acid may be produced and delivered to the central nervous system following inflammation by the action of macrophages. Kynurenic acid, however, functions as a noncompetitive agonist and interacts with the same receptor system that is affected by quinolinic acid, but kynurenic acid can also cause seizures at high levels.[16] The induction of IDO in inflammatory diseases often results in aberrant tryptophan metabolism which results in neurological disorders caused by the overproduction of quinolinic acid.

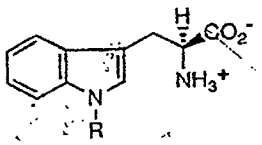
Two types of inhibitors of IDO have been reported, competitive and noncompetitive. The first competitive IDO inhibitor, 2,5-dihydro-L-phenylalanine (K_i = 230 μM), was reported by Watanabe and coworkers in 1978.[17] Since then, Cady and Sono have also reported examples of potent competitive inhibitors of IDO.[18] Their compounds were distinguished by replacement of the N-H function of the indole ring of tryptophan with N-Me, O and S functions. These inhibitors exhibited activity towards rabbit small intestine IDO with K_i values of 7-70 μM and competed with tryptophan for the active site of the ferrous-O_2 enzymatic form. The affinity (K_m) of L-tryptophan for IDO is 13 μM.[19] In contrast to indole-modified inhibitors, modification of the amino acid function of tryptophan has been shown to produce compounds which are neither substrates nor inhibitors of IDO.[18b] Hayaishi and coworkers first reported that β-carbolines were inhibitors of IDO.[20] These workers found that norharman inhibited IDO with a K_i value of 120 μM. A noncompetitive mechanism of inhibition for norharman was later established based on kinetic and spectroscopic data.[21] We have recently reported that 3-*n*-butyl-β-carboline is a very potent noncompetitive inhibitor (K_i = 3.3 μM) in human macrophage IDO systems.[22]

Since IDO depletes L-tryptophan from the amino acid pool, activation of this enzyme to high levels potentially serves to inhibit cell growth. This property can be exploited in the treatment of certain cancerous cell lines.[10,23] Infusion of interferon-γ causes an antiproliferative effect in these cells.[24] In addition, compounds which can further activate IDO may also be beneficial in the treatment of cancerous cell lines. The existence of an allosteric site on IDO has been proposed; high levels of L-tryptophan and other compounds inhibit IDO by binding to this allosteric site.[25] Morever, binding of 3-indolylethanol (tryptophol) to rabbit intestine IDO *via* this allosteric binding site resulted in a 61% activation of IDO towards tryptophan metabolism.[17] This allosteric site may be involved in the regulation of IDO or may be a site incidental to the principal function of IDO. In related work hydroquinone has been shown to induce IDO activity by 266% in the skin and by 291% in the liver of the adult male toad (*Bufo melanostictis*) *in vivo*.[26] The mechanism of the induction of IDO by hydroquinone is not known.

Results and Discussion.

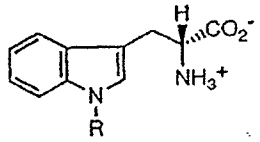
The results of this present report are intended to demonstrate some of the structural requirements of the tryptophan binding site of human monocyte/macrophage IDO for the purpose of

developing a potent inhibitor of this enzyme. Additional investigations are focused on the development of a potent effector for IDO for the treatment of cancerous cells. The following tryptophan derivatives **3-15** were evaluated for IDO inhibition.



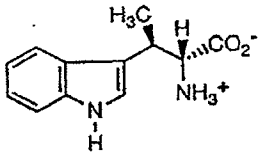
3a R = CH_3 (2S)

4a R = CH_2CH_3 (2S)

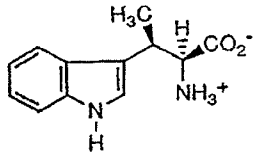


3b R = CH_3 (2R)

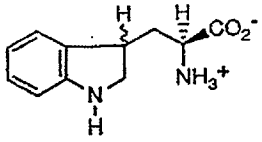
4b R = CH_2CH_3 (2R)



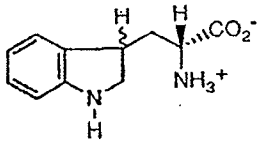
5 (2S,3S/2R,3R)



6 (2S,3R/2R,3S)



7 (2S)



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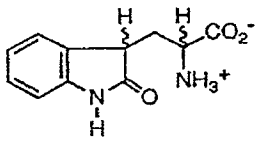

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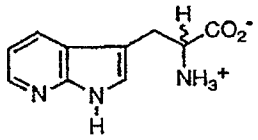

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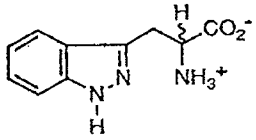

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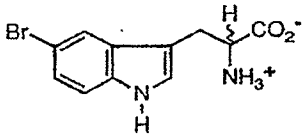

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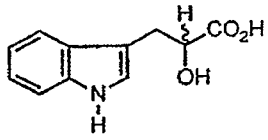

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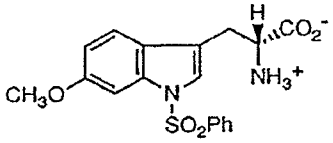

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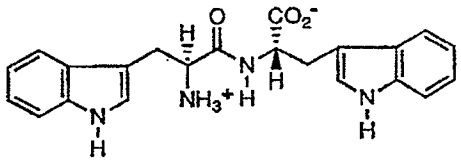

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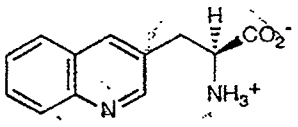
Ligands **16-28** were also investigated as inhibitors of IDO because these compounds resemble tryptophan but lack either an indole or an α-amino acid function.



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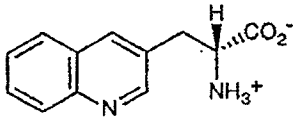

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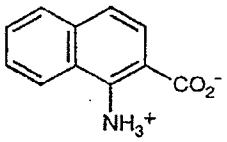

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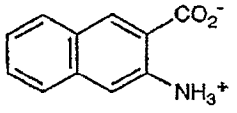

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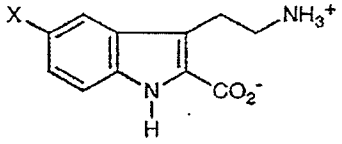
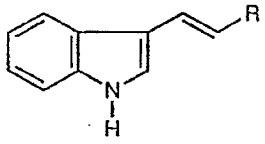


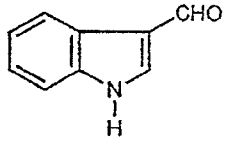
20 X = H

21 X = OCH_3



22 R = CO_2H

23 R = CO_2iPr



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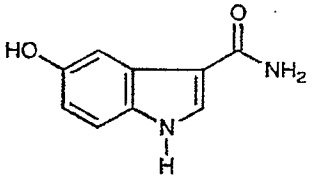

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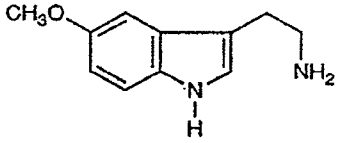

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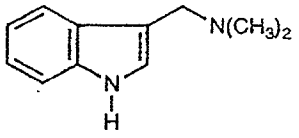

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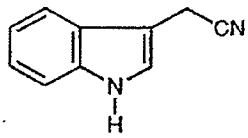

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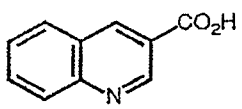
The remaining compounds **29-36** are metabolites of the kynurenine or serotonin pathways or are related compounds.



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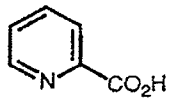

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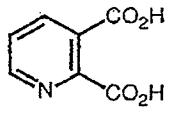

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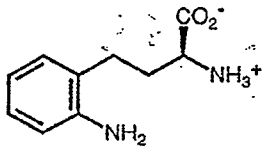

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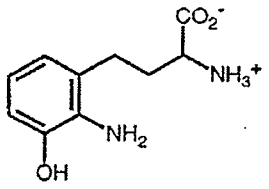

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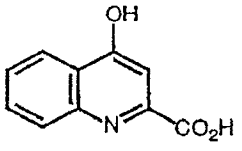

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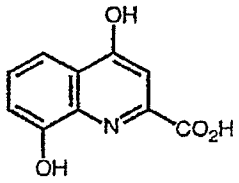

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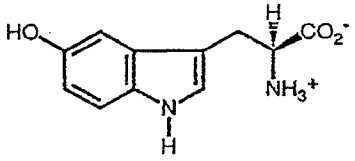

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The results of the *in vitro* competitive inhibition assays are shown in Table 1, and the results of two inhibitors **3a** and **15** are expressed as K_i values in Table 2.

Table 1. Percentage of *in vitro* Inhibition (%) of Human Monocyte/macrophage IDO by Functionalized Tryptophans and Related Compounds.

Inhibitor[a,b]	% I (0 h Preincubation)[c]	% I (2 h Preincubation)[c]
3a	52.3	62.9
3b	5.7	11.6
4a	13.5	9.9
4b	0.0	
5	0.0	-2.7
6	9.8	3.6
7	0.4	3.0
8	-2.4	-1.2
9	-38.4	-43.3
10	-1.6	
11	0.0	
12	0.0	

Table 1, continued.

Inhibitor[a,b]	% I (0 h Preincubation)[c]	% I (2 h Preincubation)[c]
13	9.7	1.4
14	3.2	28.4
16	0.0	
17	0.0	
18	-2.0	11.2
19	74.2	75.2
20	16.3	17.9
21	10.8	3.4
22	2.5	3.2
23	15.2	11.6
24	4.4	
25	7.1	
26	-43.9	
27	-6.6	
28	3.5	
29	-2.6	
30	1.5	
31	6.8	
32	-0.3	
33	-0.4	
34	1.1	
35	2.9	
36	14.0	

[a]Assay conducted with 100 μM inhibitor concentration. [b]The most active compounds are underlined.
[c]*in vitro* competitive inhibition assay reported as 100 minus percent of tryptophan metabolized.

Table 2. K_i Values for Inhibitors **3a** and **15**.

Inhibitor	K_i (μM)[a]
3a	34.0
15	147.0

[a]*in vitro* competitive binding assay results reported as K_i values.

Although both L- and D-tryptophan have been shown to be metabolized by rabbit small intestine IDO, the L isomer is metabolized 131 times faster than the D form at pH 7.[24] This suggests that the active site of IDO differentiated between the two stereoisomers. To test this hypothesis in human macrophages, optically pure L-1-methyltryptophan **3a** and D-1-methyltryptophan **3b** were prepared and evaluated as inhibitors (Table 1). As expected, the L isomer 3a (62.9 % inhibition with 2 h preincubation) was found to be a stronger competitive inhibitor of IDO *in vitro* with tryptophan as the substrate than the D form **3b** (11.6% inhibition with 2 h preincubation). The K_i value of 3a was found to be 34 μM at pH 7. The earlier report of a K_i value of 7 μM was determined with racemic 1-methyltryptophan at pH 8 on rabbit small intestine IDO.[18] The activity of the 1-ethyl analogs **4a** and **4b** (13.5% and 0%, respectively) was greatly reduced from L-1-methyltryptophan 3a. This result defines the approximate size of the substituent on the indole nitrogen of active ligands to a methyl group and further defines potent activity to the L-amino acid analogs. The weak inhibition observed for the N_a-sulfonamidotryptophan **14** further reinforces this hypothesis (size); however, the electron-withdrawing ability of the sulfonamide function may contribute to the weak activity of sulfonamide **14**. Further definition of the tryptophan binding site was accomplished with the β-methyl analogs **5** and **6** which exhibited almost no inhibition of IDO. The β-position of tryptophan can tolerate a group no larger than a methyl group for competitive inhibition. Modification of the indole 2,3-double bond provides derivatives which cannot be oxidized by IDO. Reduction of the indole 2,3-double bond of both D- and L-tryptophan furnished indolines **7** and **8** which were devoid of activity. Based on the limited amount of evidence available, this result suggests that the binding site requires a substrate with planarity at the 2,3 and 4-positions of tryptophan. The oxindole analog of tryptophan, DL-oxindolylalanine **9**, activated IDO by 43.3%. This compound represents a new class of effectors for IDO. Azatryptophans **10** and **11** which have a nitrogen atom substituted for a CH_2 group at the 2 or 7 position were inactive. This result suggests that the indole portion of tryptophan is binding in a largely lipophilic cleft. The weak inhibition of 5-bromotryptophan **12** also supports this hypothesis. In accord with earlier observations, modification of the amino acid side chain reduced the ability of the compound to inhibit IDO;[21b] the indole lactic acid **13** exhibited poor inhibition (9.7% inhibition) as previous workers had found with rabbit small intestine IDO.[22] Both 3-indoleacrylic acid **22** and 3-*i*-propyl acrylate **23**, available from unrelated work in our laboratory, were weak inhibitors, but the ester **23** was slightly more potent. The dimer L-tryptophyl-L-tryptophan **15** was also a weak inhibitor (K_i = 147 μM). This dimer **15** can be viewed as a tryptophan with substitution at the carboxylic acid function. Although dimer **15** is an amino acid, the carboxy substituent is relatively large and probably interferes with binding in the α-amino acid binding region. Surprisingly, the quinolinic amino acids **16** and **17** did not inhibit IDO despite the potential to act as either a competitive or noncompetitive inhibitor. The quinolinic amino acids **16** and **17** were chosen because of their resemblence to tryptophan, availability in both optically active forms and suspected inertness towards oxidation. The aromatic β-amino acids **18** and **19** were also evaluated as inhibitors. These amino acids were also available from unrelated work and are a test of the possible requirement for both α-amino acid and indole functions for potent inhibition of IDO. Amino acid **19** was found to be a potent inhibitor of

IDO (75.2% inhibition) while isomer **18** was a weak inhibitor of IDO. Examination of this result further demonstrates that nonindolic amino acids are capable of inhibition of IDO and that the indole N-H function is not absolutely necessary for binding at the active site. The tryptamine-2-carboxylic acids **20** and **21**, available from previous work, were weak inhibitors of IDO despite their amino acid nature. All of the commercially available indoles **24-28**, which were not α-amino acids but contain a side-chain function that is capable of hydrogen bonding, were found to be inactive except for 5-methoxytryptamine **26** which proved to be a relatively potent effector for IDO (43.9% activation of IDO). Compounds **29-36** were investigated as IDO inhibitors in an attempt to discover possible regulation of IDO by metabolites of the serotonin pathway and metabolites of the later stages of the kynurenine pathway. None of these metabolites or related compounds **29-36** were active except for 5-hydroxytryptophan **36** which inhibited the production of kynurenine by 14%. The inactivity of 3-quinoline carboxylic acid **29** compared to 3-amino-2-naphthoic acid **19** parallels the difference in activity between quinoline-3-alanines **16** and **17** and tryptophan **1** or 1-methyltryptophan **3**. The largely lipophilic cleft of IDO which binds indoles and naphthalenes excludes the binding of the quinolines investigated here.

To these authors' knowledge, the ligands L-(-)-1-methyltryptophan **3a** and 3-amino-2-naphthoic acid **19** are the most potent competitive inhibitors for human monocyte/macrophage IDO reported to date. These ligands should provide new leads for the synthesis of even more potent competitive inhibitors of this enzyme for the treatment of aberrant tryptophan metabolism in immunological diseases. The potent activity of naphthoic acid **19** indicates that the tryptophan indole unit binds in a largely lipophilic cleft and that the indole N(1)-H function is not necessary for potent activity at the tryptophan binding site. Furthermore, stereoselectivity of binding at the competitive binding site of IDO was clearly observed in this study. In addition, 3-*n*-butyl-β-carboline was previously found to be the most potent noncompetitive inhibitor (allosteric site) of this enzyme.[1,22] Both DL-oxindolylalanine **9** and 5-methoxytryptamine **26** were found to be the two most potent effectors of human monocyte/macrophage IDO reported to date and may find a use in the treatment of certain lines of cancerous cells.

Experimental.

Enzyme assays were conducted as previously described.[22] Microanalyses were performed on an F and M Scientific Corp. Model 185 carbon, hydrogen and nitrogen analyzer. Melting points were taken on a Thomas-Hoover melting point apparatus and are reported uncorrected. ^{1}H and ^{13}C NMR spectra were obtained with a Bruker WM250 or a GE GN500 spectrometer. Infrared spectra were recorded on a Nicolet MX-1 FT-IR spectrometer. Mass spectral data (EI/CI) were obtained with a Hewlett-Packard 5985B GC-mass spectrometer. Thin layer chromatography was performed with E. Merck Brinkman UV active silica gel (Kieselgel 60 F254 on plastic), and the plates were visualized with UV light. Dichloromethane was dried over P_2O_5 and distilled under an atmosphere of nitrogen. The following compounds were obtained from Aldrich Chemical Co. and used without further purification: DL-5-bromotryptophan **12**, DL-indolelactic acid **13**, 3-amino-2-naphthoic acid **19**, 3-

indoleacrylic acid **22**, indole-3-carboxaldehyde **24**, 5-methoxytryptamine **26**, gramine **27**, 3-indolylacetonitrile **28**, 3-quinoline carboxylic acid **29**, picolinic acid **30**, quinolinic acid **31**, 3-hydroxykynurenine **33**, kynurenic acid **34**, xanthurenic acid **35**, L-5-hydroxytryptophan **36** and (R)-(+)-α-methylbenzylisocyanate. The following compounds were purchased from Sigma Chemical Co. and used as received: DL-7-azatryptophan **10**, L-tryptophyl-L-tryptophan **15**, L-kynurenine **32**. The ligand, 5-hydroxyindole-3-acetamide **25**, was purchased from ICN Biomedicals, Inc. and used as received. The following compounds were prepared by literature methods: L-(-)-1-methyltryptophan **3a**[27], D-(+)-1-methyltryptophan **3b**[27], L-(-)-dihydrotryptophan **7**[28], D-(+)-dihydrotryptophan **8**[28], DL-oxindolylalanine **9**[29], tryptamine-2-carboxylic acid **20**[30] and 5-methoxytryptamine-2-carboxylic acid **21**[30].

L-(-)-1-Ethyltryptophan (4a). A 5 L three neck flask equipped with a mechanical stirrer and dry ice condenser was cooled in a dry ice/EtOAc bath and filled with liquid ammonia (4 L). Metallic sodium (23.0 g, 1.0 mmol) and $FeCl_3$ (0.65 g, 4 mmol) were added with stirring. After 1 h, L-tryptophan (100 g, 0.49 mmol) was added in one portion to the cooled mixture, and the resultant slurry was stirred for 10 min. Iodomethane (101.33 g, 0.65 mmol) was added dropwise over a 10 min period, and the resultant mixture was stirred for 1 h. The cooling bath was removed, and the ammonia was allowed to evaporate in the fume hood for 12 h. The residue which remained was dissolved in hot H_2O (400 mL, 65 °C) and filtered. The pH of the filtrate was adjusted to 5 with glacial acetic acid. Precipitation of L-(-)-1-ethyltryptophan occurred upon cooling. The crude material was recrystallized from H_2O, filtered and washed with copious amounts of H_2O to give 77.1 g of amino acid **4a** as a solid. Purification of amino acid **4a** was accomplished by recrystallization from absolute EtOH followed by drying *in vacuo*: mp 197-199 °C; $[\alpha]_D^{24}$ -14.4° (c 1.0, HOAc); IR (KBr) 3426 (br), 2976 (br), 1615, 1473, 1396, 1325, 1241, 1213, 1140, 1071, 1015, 856, 726, 672; ^{1}H NMR (DMSO-d6) δ 8.32 (br s, 3 H), 7.58 (d, J = 7.8 Hz, 1 H), 7.44 (d, J = 8.0 Hz, 1 H), 7.31 (s, 1 H), 7.14 (t, J = 7.0 Hz, 1 H) 7.02 (t, J = 7.5 Hz, 1 H), 4.15 (q, J = 7.3 Hz, 2 H), 3.42 (q, J = 7.0 Hz, 1 H), 3.26 (dd, J = 7.0 Hz, 2 H), 1.34 (t, J = 7.3 Hz, 3 H); MS(CI, CH_4) m/e (relative intensity) 233 (M+1, 7), 216 (91), 187 (12), 158 (100), 143 (9), 130 (35), 115 (7), 102 (16); Anal. Calcd. for $C_{13}H_{16}N_2O_2$: C, 67.22; H, 6.94; N, 12.06. Found: C, 66.46; H, 7.10; N, 11.80.

D-(+)-1-Ethyltryptophan (4b). The procedure for the preparation of L-(1)-ethyltryptophan **4a** described above was followed. The physical and spectral properties of amino acid **4b** were identical to those of amino acid **4a** except for the optical rotation: $[\alpha]_D^{24}$ +14.4° (c 1.0, HOAc); Anal. Calcd. for $C_{13}H_{16}N_2O_2$: C, 67.22; H, 6.94; N, 12.06. Found: C, 66.65; H, 7.18; N, 11.83.

1-Benzenesulfonyl-6-methoxy-D(+)-tryptophan (14). A mixture of 1-benzenesulfonyl-6-methoxy-D(+)-tryptophan ethyl ester (198 mg, 0.49 mmol)[31] and 6 N HCl (10 mL) was heated to reflux for 12 h. The resultant mixture was cooled to room temperature and concentrated *in vacuo*. A solution of 50% EtOH-H_2O (20 mL) was added to the crude product followed by 2 N NaOH to attain

pH 5. The white solid that precipitated was filtered and recrystallized from H_2O to give 178 mg (96%) of amino acid **14**; mp 222-223 °C (dec); IR (KBr) 3425 (br), 2976 (br), 1619, 1406, 1372, 1344, 1175, 1113 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 7.95 (d, J = 8.2 Hz, 2 H), 7.67 (t, J = 8.2 Hz, 1 H), 7.58 (t, J = 8.2 Hz, 2 H), 7.53 (s, 1 H), 7.49 (d, J = 8.6 Hz, 1 H), 7.33 (d, J = 2.2 Hz, 1 H), 6.89 (dd, J = 2.2, 8.6 Hz, 1 H), 3.79 (s, 3 H), 3.43 (dd, J = 4.2, 8.4 Hz, 1 H), 3.17 (dd, J = 4.2, 15.6 Hz, 1 H), 2.89 (dd, J = 8.4, 15.6 Hz, 1 H); MS(EI) 374 (M^+, 5), 301 (12), 300 (59), 159 (100), 145 (13), 141 (19), 117 (15). Anal. Calcd. for $C_{18}H_{19}N_2O_5S$: C, 57.59; H, 5.10; N, 7.46. Found: C, 57.12; H, 4.69; N, 7.32. $[\alpha]_D^{24}$ +18.4° (c 0.25, HOAc).

1-Amino-2-naphthoic acid (18).[33] A 20% aq. NaOH solution (50 mL) was added to a solution of 1-amino-2-cyanonaphthalene (5 g, 30 mmol) in ethanol (150 mL). The mixture was heated at reflux for 10 h, cooled to room temperature, and the ethanol was removed *in vacuo*. The aqueous solution was brought to pH 6.5 with concentrated HCl which gave a white precipitate. The solid was collected by filtration, washed with H_2O and dried under vacuum to provide 4.2 g (85%) of amino acid **18**: mp 270 °C (dec), lit. mp 198-199 °C[33]; IR (KBr) 3161, 3156, 3018, 2896, 2867, 2792, 1677, 1617, 1442 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.90 (d, J = 7.4 Hz, 1 H), 8.01 (d, J = 8.7 Hz, 2 H), 7.86 (d, J = 8.7 Hz, 1 H), 7.66-7.77 (m, 2 H); MS(EI) m/e 187 (M^+, 16), 141 (19).

***i*-Propyl 2-Indoleacrylate (23).** A solution of N_b-diphenylmethyltryptophan *i*-propyl ester[32] (0.71 g, 1.7 mmol) and (R)-(+)-α-methylbenzylisocyanate (0.25 g, 1.7 mmol) in anhydrous CH_2Cl_2 (35 mL) was stirred at room temperature. The reaction was monitored by TLC (10% EtOAc-benzene) until the starting material was consumed. The reaction mixture was concentrated *in vacuo* to give 310 mg (79%) of white, crystalline solid **23**: mp 174-179 °C (dec), ^1H NMR (250 MHz, DMSO-d_6) δ 7.4-7.1 (m, 5 H), 6.24 (d, 1 H), 4.8-4.6 (m, 3 H), 1.29 (s, 3 H), 1.26 (s, 3 H); MS(EI) m/e 411 (M^+ - 1, 1), 331 (1), 282 (1), 229 (30), 167 (99), 165 (28), 152 (17), 130 (100), 105 (67), 103 (17).

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